



Atty Dkt. No.:CLON-060
USSN:09/960,716

**DECLARATION
OF GRIGORIY TCHAGA
UNDER 37 C.F.R. § 1.132**

Address to:
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Attorney Docket Confirmation No.	CLON-060 4277
First Named Inventor	Grigoriy Tchaga
Application Number	09/960,716
Filing Date	September 21, 2001
Group Art Unit	1641
Examiner Name	Ann Y Lam
Title:	HIGHLY SENSITIVE PROTEOMIC ANALYSIS METHODS, AND KITS AND SYSTEMS FOR PRACTICING THE SAME

Dear Sir:

1. I, Grigoriy Tchaga, declare and say I am a resident of the State of California. My residence address is 5322 Yarmouth, Newark, CA 94560.
2. I hold a Ph.D., which I received from Uppsala University, Uppsala, Sweden in 1994. I am skilled in the fields of Biochemistry and Molecular Biology. I am a co-inventor of the invention claims of the above-referenced patent application.
3. I have reviewed the relevant portions of the current Office Action mailed December 8, 2006, in the above-referenced application. I understand that claims of the above-referenced patent application are rejected under 35 USC §103(a) on the grounds that they are unpatentable over the combined teachings of the following references:
 - Margherita et al. (4,111,656) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596);
 - Margherita et al. (4,111,656) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596), Schoemaker et al. (4,837,167) and Pronovost et al. (5,773,234);
 - Margherita et al. (4,111,656) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596) and Wohlstadter et al. (6,207,369);

- Margherita et al. (4,111,656) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596), Schoemaker et al. (4,837,167), Pronovost et al. (5,773,234) and Wohlstadter et al. (6,207,369);
- Velander et al. (5,328,603) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596); and
- Velander et al. (5,328,603) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596), Schoemaker et al. (4,837,167), Pronovost et al. (5,773,234) and Wohlstadter et al. (6,207,369).

4. In the Office Action of December 8, 2006, the Examiner stated that "If the sample *without* the metal chelating polysaccharide did *not* have any metal ion chelator... then the difference in the background fluorescence may be due to the lack of any metal ion chelator as opposed to any differences between the metal ion chelating polysaccharide and a non-polysaccharide metal ion chelator..." Through this and other statements in the present Office Action, the Examiner is making clear that it one of skill in the art would expect that replacing a metal ion chelating agent for another in an array assay binding buffer would not significantly reduce the background fluorescence in the assay. I concur with this position.

5. This Declaration provides evidence of unexpected results obtained by employing the methods of the claimed invention. Specifically, the data shown in Exhibit A and described herein demonstrate that the inclusion of a metal ion chelating polysaccharide as claimed (i.e., apple pectin) significantly reduces background in an array-based analyte detection assay over other known metal ion chelating agents. These results meet criteria for establishing that the claimed invention provides unexpected results as espoused by the Examiner (and described above). Therefore, it is my contention that practicing the claims of the present application produces unexpected results.

6. The following experiments were conducted by me or under my direction.
7. Exhibit A shows the results of six array-based analyte detection assays. The assays were performed under standard assay conditions (as described in detail below), with one performed as claimed in the subject invention (i.e., in which the binding buffer contains apple pectin). The data in Exhibit A clearly demonstrate that the background fluorescence present on the array contacted with the sample containing 0.2% apple pectin (panel F) is significantly reduced as compared to those without apple pectin (i.e., (-) Control, panel A) as well as those in which apple pectin is replaced with 1% of the specified organic acid metal ion chelating agent (i.e., adipic acid, maleic acid, malonic acid, and succinic acid; panels B to E, respectively). The average signal to background ratio (Sig/BG) for the region in the boxed area is shown to the right of each array image. The boxed region corresponds to the first 6x8 quadrant of features on each array. The fold increase in signal to background ratio in the apple pectin containing sample ranges from 13.7 fold to 17.4 fold.

8. Excluding the metal ion chelating component, the incubation buffer for each array assay performed in Exhibit A (i.e., the buffer employed for the sample/array contacting step of the assay) contained the following:

- 5ug Cy3-labeled HeLa protein extract;
- 5ug Cy5-labeled HeLa protein extract;
- 10mM Tris;
- 150mM NaCl;
- 5% Tween 20; and
- 5% Pluronic 69 (pH 7.4).

As noted above, each of the organic acid metal ion chelating agents were added to a final concentration of 1% and the apple pectin was added a final concentration of 0.2% (keeping the final pH of all samples at 7.4). Arrays were contacted to their

respective incubation buffers, incubated, washed, and read by the array reader using identical conditions. As such, the only variable in the assays was the presence (or absence) of one of the metal ion chelating agents under study (i.e., the organic acid metal ion chelating agents and apple pectin).

9. The data provided herein demonstrate that the inclusion of a metal ion chelating polysaccharide provides an unexpected reduction in the background fluorescence in array based analyte detection assays over other metal ion chelating agents.
10. I, Grigoriy Tchaga, hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such will false statements may jeopardize the validity of the application or any patent issuing thereon.

March 6, 2007
Date

Grigoriy S. Tchaga, Ph.D.
Grigoriy Tchaga, Ph.D.

Attachments: Exhibit A, 1 page

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